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Alberta Environmental Centre

Series on Inhalation Toxicology

1. Morphological Observations in Rats Exposed for Six Hours to an Atmosphere of 0, 56, or 420 mg m^{-3} Hydrogen Sulphide.

Alberta
ENVIRONMENTAL CENTRE

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ALBERTA ENVIRONMENTAL CENTRE
SERIES ON INHALATION TOXICOLOGY

1. MORPHOLOGICAL OBSERVATIONS IN RATS EXPOSED FOR SIX HOURS TO AN ATMOSPHERE OF 0, 56 OR 420 mg m^{-3} HYDROGEN SULPHIDE

Alberta Environmental Centre

Vegreville, Alberta T0B 4L0

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STUDY TEAM

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ABSTRACT

Forty eight male Long Evans rats were exposed to nominal concentrations of 0, 56 or 420 mg m^{-3} (actual 0, 57 ± 15 or 420 ± 1.4 mg m^{-3}) hydrogen sulphide for six hours. Weight loss was observed in all rats exposed to hydrogen sulphide, as was agitation, hypoaesthesia, panting and lacrimation. All rats exposed to 300 ppm died within the six hour exposure period. Necrosis of the nasal epithelium was more marked in the intermediate (sectors 2, 3) than the most rostral (sector 1) and most caudal (section 4) parts of the nasal cavity. The lateral aspects of the nasal turbinates revealed more necrosis when compared to the median aspects, especially the epithelium covering the nasal septum. Mild pulmonary oedema was observed in all animals exposed and killed by 420 mg m^{-3} hydrogen sulphide and in those treated with 56 mg m^{-3} and killed at the end of the exposure. Rats exposed to 56 mg m^{-3} did not show pulmonary oedema at 18 or 42 hours post exposure. The oedema had a perivascular distribution, and fluid was rarely seen within the alveoli.

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1. INTRODUCTION

Hydrogen sulphide is a colourless gas having, at low concentrations, the characteristic odour of rotten eggs. The gas occurs naturally in coal, natural gas, oil, volcanic gases, sulphur springs and lakes, and is a product of the anaerobic decomposition of sulphur-containing organic matter. It is a by-product of the production of coke, manufacture of gases from coal, production of viscose rayon, the kraft process for producing chemical pulp from wood, and desulphurization of sour natural gas. The occupational hazards of exposure to hydrogen sulphide are well known (National Research Council of Canada, 1981).

People in a number of agricultural communities in Alberta close to sulphur extraction gas plants, and who have been personally affected by emissions, demonstrate an extremely high level of concern for the effects of gas plant pollution (Environment Conservation Authority, 1973). Well blowouts may release hydrogen sulphide into the atmosphere as happened, for example, in the Camrose area in 1973, the Drayton Valley-Lodgepole area in 1977 and again in 1982 (New Norway Scientific Committee, 1974; Energy Resources Conservation Board, 1978; Lodgepole Blowout Inquiry, 1984). The last example was the most extensive; the highest concentration recorded away from the blowout

site was 32 mg m^{-3} (23 ppm) at a location 13 km southeast of the blowout site, the maximum concentration at two towns 20 km to the east and northeast was 20 mg m^{-3} (14.5 ppm), and the maximum concentration at Edmonton, some 140 km to the east-northeast, was 0.7 mg m^{-3} (0.5 ppm) (Lodgepole Blowout Inquiry, 1984). In 1984, three (9.4%) of 32 industrial fatalities in Alberta were due to hydrogen sulphide toxicity (Occupational Health & Safety, 1984).

The clinical signs and symptoms of hydrogen sulphide poisoning include nausea, vomiting, headache, dizziness, loss of appetite, gastrointestinal disturbance, fatigue, muscular weakness and nervous disorders. Hydrogen sulphide is a chemical asphyxiant as well as an irritant. Inhalation of high concentrations (greater than 700 mg m^{-3}, 500 ppm) of this gas results in paralysis of the respiratory centre of the brain (Haggard, 1925; Yant, 1930; Ahlborg, 1951; Milby, 1962; Poda and Aiken, 1966; Jones, 1975). In cases of acute toxicity where instantaneous deaths have occurred, post-mortem examination revealed hemorrhagic pulmonary oedema, petechial hemorrhages in the pleura, and generalized visceral congestion (Adelson and Sunshine, 1966; Simson and Simpson, 1971).

Although there are some scattered reports in the literature on the pathology of experimental hydrogen sulphide toxicity, the precise effects of this gas on the respiratory membrane, specifically on the nasal mucosa, have been poorly characterized (CIIT/ToxiGenics, 1983a, 1983b; Beauchamp et al, 1984). The mandate of the Alberta Environmental Centre is to solve environmental problems for provincial

government departments (Alberta Environmental Centre, 1983). The Inhalation Toxicology research program of the AEC was developed in response to concerns for the effects of toxic or potentially toxic gases, vapours and particulates on animals and, by extrapolation, man. The present study reports the initial histopathological findings on rats exposed to a concentration of 0, 56 or 420 mg m^{-3} hydrogen sulphide for six hours, with particular emphasis on the lesions observed in the nasal mucosa.

The majority of the examined literature reports the concentrations of hydrogen sulphide in ppm, with occasional mention of the relevant SI units, namely mg m^{-3}. For hydrogen sulphide 1 ppm is equivalent to 1.4 mg m^{-3}.

2. MATERIALS AND METHODS

2.1 ANIMALS, CARE AND HOUSING:

Forty eight (48) male Long Evans rats were obtained from commercial sources (Charles River Inc., St. Constant, Quebec). The animals were housed individually in stainless steel mesh-bottomed cages and kept in an environmentally controlled room with a temperature of 22 ± 2°C, relative humidity of 50 ± 20%, and a photo-period of 12 h light and 12 h dark. A certified laboratory rodent feed (Purina Laboratory Rodent Chow #5002, St. Louis, Missouri) and reverse osmosis water were provided ad libitum to the rats. After

a two week acclimatization period, the animals were randomly divided into four groups of 12 rats: Group I (room control), Group II (chamber control), Group III (56 mg m^{-3} hydrogen sulphide) and Group IV (420 mg m^{-3} hydrogen sulphide). Body weights were taken weekly during the acclimatization period and daily on the day of exposure and thereafter. The guidelines of the Canadian Council of Animal Care (1980) were followed throughout all phases of the study. The experimental design and the time of euthanasia after exposure are shown in Table 1.

2.2 GAS EXPOSURE SYSTEM

The system which consisted of sources of air and hydrogen sulphide, flow controllers, a mixing device, exposure chambers, vacuum pump and scrubber, is summarized diagrammatically in Figure 1. The exposure chambers were constructed of a clear, acrylic cylinder, with two removable stainless steel cones, and have a volume of 69.3 ± 0.2 L. Each chamber held three circular stainless steel mesh cages, holding 4 rats per cage in individual compartments. Hydrogen sulphide, 99.5% C.P., (Matheson Gas Products, Edmonton, Alberta) contained in a gas cylinder, passed first through a two-stage regulator and was maintained at a pressure of 413.7 x 10^3 Pa. It then passed through a flowmeter, and mixed with a supply of filtered air. The rates of flow of hydrogen sulphide and air were adjusted to maintain the target concentration in the test chambers, and these

values used to calculate the nominal concentration. The airflow in the chamber was 17 L min^{-1}, or approximately 15 air changes per hour. Exhaust air from the test chambers was passed through a portable fume scrubber (Mystaire, Model HS-7, Plainview, New York) containing an 6% sodium hypochlorite solution, before release into the atmosphere.

2.3 HYDROGEN SULPHIDE MONITORING SYSTEM

The test chambers were maintained at a (negative) pressure of -74.6 Pa, and the control chamber at a (positive) pressure of 124.3 Pa for the duration of the exposures. A gas chromatographic monitoring system was employed to analyze air samples obtained by automatic sampling methods. Automatic chamber monitoring was achieved using a pneumatically operated sampling system (Valco Instruments, Houston, Texas). This system operated using a continuous flow through a 12 port multiple stream complex in conjunction with a gas sampling valve. The 12-port valve was connected to the gas chromatograph via the carrier inlet and analytical column. A calibrated sample loop was used to control the injection volume. A digital valve controller determined the frequency of injections and started the analogue to digital conversion. Each test chamber was sampled approximately four times per hour. Each chamber was fitted with three sample lines, one in the supply line and two in the chamber. Control atmospheres were also sampled approximately four times per hour. Exposure room air was

monitored continuously for hydrogen sulphide using a Hydrogen Sulphide Monitor (Interscan Corp., Model R-17, Chatsworth, California). The gas chromatograph and monitoring system instruments were located in a room adjacent and isolated from that of the exposure chambers. The samples were analyzed for hydrogen sulphide using a Gas Chromatograph (Hewlett Packard, Model 5790A, Edmonton, Alberta), with a Sample/Event Controller Module (Hewlett Packard, Model 19400A), and an Integrator (Hewlett Packard, Model 3390A). Analysis was achieved using a 2 x 0.003 m, 80/100 mesh, silanized porous ethylvinyl benzenedivinyl-benzene copolymer bead column (Poropak QS™, Chromatographic Specialities, Brockville, Ontario) and Flame Photometric Detector (Hewlett Packard).

2.4 ACCLIMATIZATION AND TRAINING OF RATS TO CHAMBERS

Rats from groups II, III and IV were acclimatized to the noise and confinement of the exposure chambers before exposure to hydrogen sulphide. The rats in these three groups were trained for six hours per day for four days. The animals were removed from their individual home cages and placed into the circular wire mesh cage holding compartments. Three of these cages (12 rats) were placed into each of the three exposure chambers. The supply air (compressed air) was turned on and the three exposure chambers sealed. The vacuum pumps were adjusted to hold each treatment chamber at a pressure of -74.6 Pa, and the control chamber at +124.3 Pa. The portable fume scrubber

pump was turned on and the animals were left for six hours to acclimate to the noise levels and the exposure chambers. At the end of this time they were returned to the appropriate home cages. After the first two hours on the first training day the animals calmed down and returned to normal behaviour such as grooming and sleeping.

2.5 HYDROGEN SULPHIDE EXPOSURE

The rats were placed into the exposure chambers. Chambers 1 and 2 were in a fume hood. Chamber 1 was metered to receive a nominal concentration of 420 mg m^{-3} hydrogen sulphide, actual 420 ± 1.4 mg m^{-3} (Mean ± SE), range 417-420 mg m^{-3}, and Chamber 2 a nominal concentration of 56 mg m^{-3}, actual 57 ± 15 mg m^{-3}, range 20 - 84 mg m^{-3}. Chamber 3 received only air. All groups were exposed simultaneously to either hydrogen sulphide or air for six consecutive hours.

2.6 EUTHANASIA AND COLLECTION OF SAMPLES

The rats were anesthetized with 3-5% halothane (MTC Pharmaceutical, Mississauga, Ontario), the abdominal cavity opened and the abdominal aorta severed to ensure rapid death by exsanguination. The ribs were cut with scissors and the thoracic viscera exposed and examined for gross morphological changes. Fixation of the nasal epithelium was done by intravascular perfusion via left ventricle with

Karnovsky's fixative (610 mOsm) following the technique described by Monteiro-Riviere and Popp (1984). The lungs were removed en bloc and the trachea connected to a fixation apparatus containing outlets for 16 lung attachments and a reservoir for fixative. The lungs were perfused with 25% Karnovsky's fixative (610 mOsm) at room temperature with a flow of 30 cm water pressure for 20-22 h. The nasal cavity was flushed with 0.9% sodium chloride solution (Travenol, Mississauga, Ontario) to eliminate excess mucus. Four different cross sections of nasal structure were made following the technique described by Monteiro-Riviere and Popp (1984). In addition, the following tissues were taken and fixed in 10% buffered formalin for histopathological evaluation: brain, spinal cord, pituitary gland, cervical lymph node, thymus, thyroid, heart, salivary glands, tongue, esophagus, stomach, ileum (Peyer's patches), caecum, liver, spleen, adrenals, kidneys, bone (left femur), and striated muscle. The eyes and conjunctiva were fixed in Bouin's solution.

3. RESULTS

3.1 CLINICAL SIGNS

For a period of two hours after gas exposure the rats of group III (56 mg m^{-3} hydrogen sulphide) were agitated, and showed a moderate degree of hypoaesthesia, panting and lacrimation. The rats of group IV (420 mg m^{-3} hydrogen sulphide) remained agitated throughout the

exposure period and were severely dyspneic until death. Rats of group II (chamber control) had no significant clinical signs and their behaviour was similar to animals of group I (room control).

3.2 MORTALITY

All rats (n=12) of group IV (420 mg m^{-3} hydrogen sulphide) died close to the end of the 6 hour exposure period. The specific time of death in this group was not known, however all animals visible through the sash of the fume hood were alive after five hours of hydrogen sulphide exposure. None of the rats exposed to 56 mg m^{-3} hydrogen sulphide or air died during the 42 hour post-exposure period.

3.3 BODY WEIGHTS

The daily weight gain during the acclimatization period was similar in all four groups. There was a considerable loss of body weight in groups III and IV after six hours of exposure to hydrogen sulphide. Rats in group II demonstrated a weight loss also, although not to the degree of groups III and IV (Table 2).

3.4 GROSS PATHOLOGY

No macroscopic lesions were observed in any animals in Groups I, II or III. In contrast, all rats in Group IV had froth in the upper

airways, the lungs were markedly congested, haemorrhagic and failed to collapse when the thoracic cage was opened. No other gross lesions were observed.

3.5 HISTOPATHOLOGY

Acute necrosis was present in the nasal epithelium of all rats exposed to and killed by the 420 mg m^{-3} of hydrogen sulphide (Table 3). Rats exposed to 56 mg m^{-3} of hydrogen sulphide had similar lesions in the nasal epithelium but to a much lesser degree (Table 3). The necrosis of the nasal epithelium in both groups exposed to hydrogen sulphide had a specific distribution within the nasal cavity. The most rostral [sector 1] and most caudal [sector 4] parts of the nasal cavity were considerably less affected than the intermediate parts [sectors 2, 3]. Furthermore, the intermediate parts of the nasal cavity were also affected in a selective pattern. The lateral aspects of the nasal turbinates revealed more severe necrosis compared to the medial aspects, especially the epithelium covering the nasal septum. The epithelium of rats exposed to 420 mg m^{-3} revealed also some degree of autolysis. Three rats in Group I and one in Group II had a focal erosive lesion in the nasal mucosa (Table 3). The ciliary epithelium in the affected areas was characterized by superficial, well circumscribed erosion with localized exudation of neutrophils, forming a plaque-like lesion on the surface of the epithelium. This type of lesion was well

localized, restricted to one per affected rat and involving either sector 1 or sector 2 of the nasal cavity.

Mild pulmonary oedema was observed in all animals exposed to, and killed by, 420 mg m^{-3} hydrogen sulphide, and in those treated with 56 mg m^{-3} and sacrificed at 0 hours post-exposure. The rats exposed to 56 mg m^{-3} and sacrificed at 18 and 42 hours did not reveal pulmonary oedema (Table 4). The oedema in the lungs had a largely perivascular distribution and fluid was rarely seen within the alveoli. The lungs of rats killed by 420 mg m^{-3} hydrogen sulphide had some fibrin-like fibrils in the alveoli and some cellular desquamation from the bronchiolar walls.

A large number of rats had lymphorrhexis in the thymus or lymph nodes or both tissues, but not in the spleen or bone marrow (Table 5). Since lymph nodes were not available for histological evaluation from each rat, the presence of lymphorrhexis was determined in either thymus or lymph nodes or both tissues. This lesion in lymphoid organs was present in all experimental groups, however, there was a tendency for higher frequency and severity of presentation in the rats exposed to hydrogen sulphide, particularly in those exposed to 420 mg m^{-3} (Table 5).

Several incidental findings not related to treatment were observed in rats of all four experimental groups (Table 6). The most remarkable of these lesions were the focal erosive rhinitis observed in the control groups and described above, and the focal hepatic necrosis affecting two rats in Group III and one rat in Group IV

(Table 6). This lesion was well demarcated and located below the hepatic capsule. The affected hepatocytes appeared swollen with hypereosinophilic cytoplasm and nuclear pyknosis with minimal inflammatory response. One rat of the room control group revealed a single well demarcated nodule of hyperplasia in the exocrine pancreas with moderate interstitial fibrosis. A rat belonging to Group II had a single focal area of mineralization of a Peyer's patch, however, the surrounding intestinal mucosa appeared intact. Discrete hyperplasia of the prostatic acini was observed in one rat of Group II and in two animals of Group IV. One rat of Group III had a well localized area of moderate neutrophilic infiltration with Zenker's degeneration of muscle fibers in striated muscle. Histologic lesions were not observed in the brain tissues of any of the experimental groups.

4.0 DISCUSSION

Lacrimation and respiratory distress observed in the rats during the six hour exposure are consistent with those of a highly irritant gas such as hydrogen sulphide. Eye injury in man resulting from exposure to hydrogen sulphide under natural conditions has been well documented in the literature (Burnett *et al*, 1977; Beauchamp *et al*, 1984). Corneal oedema and necrosis have been reported in rats kept in an atmosphere of 1820 mg m^{-3} (1300 ppm) hydrogen sulphide for 10 minutes or 76 mg m^{-3} (54 ppm) for three hours (Michal, 1950). In

contrast, histological evidence of corneal or conjunctival abnormalities were not observed in rats for up to 42 hours after exposure to 56 mg m^{-3} for six hours in the present study. The lack of ocular lesions in our experiment was likely dose or time related since severe eye irritation was clinically observed during and after hydrogen sulphide exposure. In the present study lacrimation was noticeable at 56 mg m^{-3}, in contrast to a previous report in which 76 mg m^{-3} was reported as the lower threshold for eye irritation (Michal, 1950).

Lymphoid cell necrosis was possibly a stress-induced lesion caused by the environment to which rats were subjected during exposure. More work is required to validate these observations, especially for rats exposed to high concentrations of hydrogen sulphide, since this group may have already had some degree of autolysis.

Death of the rats exposed to the high concentration of hydrogen sulphide was unexpected since the LC_{50} for hydrogen sulphide was reported to be 622 mg m^{-3} (444 ppm) (Tansy et al, 1981). However, it should be noted that this value was calculated for Sprague-Dawley rats exposed to hydrogen sulphide for four hours, whilst in our study the animals were exposed for a period of six hours. Since the rats exposed to 420 mg m^{-3} were observed to be alive at the end of the four hour exposure, it is likely that the two additional hours had a significant cumulative toxic effect despite the fact that the rats were exposed to 202 mg m^{-3} less than that reported in the LC_{50} study. In addition, pharmacokinetic differences have been reported

between the Sprague-Dawley and Long-Evans strains of rat (Taylor et al, 1984). The lower gain in body weight by the rats exposed to hydrogen sulphide was consistent with reports in the literature (CIIT/Toxigenics, 1983a, 1983b) and probably reflected, to some degree, the general malaise present in the animals during and shortly after the inhalation of hydrogen sulphide. Nonetheless, the complete survival of the rats exposed to the lower dose corroborated the general observation that in spite of the highly irritative properties of hydrogen sulphide, the survivors fully recover, without any noticeable residual signs of toxicity.

The lack of gross lesions after exposure to 56 mg m^{-3} and the haemorrhagic changes in the rats killed by the exposure to a high concentration of hydrogen sulphide are consistent with previous reports (O'Donaghue, 1961). The presence of foamy fluids in the lungs, trachea and nasal cavity of rats exposed to 420 mg m^{-3}, and mild histologic accumulation of fluid in the lungs of those animals exposed to 56 mg m^{-3} and sacrificed at zero hours post exposure, were undoubtedly good indicators of the oedematogenic properties of hydrogen sulphide on the respiratory tract. Based on the morphological findings, it is difficult to determine the precise pathophysiological origin of the oedema in the lungs. Nonetheless there could be several hypothetical explanations.

First, it is possible that the accumulation of fluids in the lungs following hydrogen sulphide inhalation was the result of direct injury produced by the gas on the respiratory membrane, especially on the

pulmonary vascular bed. Second, based on the literature, hydrogen sulphide has a remarkable effect upon the respiratory centers located in the brain stem (Evans, 1967), and perhaps pulmonary oedema might have resulted from an indirect effect of anoxia on the pulmonary vascular endothelium, resulting from preceeding respiratory paralysis. Finally, it is not possible to rule out if the oedema was cardiogenic in origin, since experimental studies have also demonstrated that sulphides, by-products of hydrogen sulphide metabolism, have a direct effect on vasomotor centers in the nervous system (Beauchamp et al, 1984). Activation of vasomotor centers by sulphide metabolites result in bradycardia and increased blood pressure (Evans, 1967; Kosmider et al, 1967). Whether or not pulmonary oedema in individuals exposed to hydrogen sulphide is neurogenic, inflammatory or cardiogenic in origin or a combination of all three, remains to be elucidated.

Necrosis of the nasal epithelium reflected severe toxicity of hydrogen sulphide to the upper respiratory epithelium even when the concentration was as low as 56 mg m^{-3}. Previous investigations did not report an effect of hydrogen sulphide on the nasal mucosa (O'Donoghue, 1961; Tansy et al, 1981). The difference in the severity of necrosis of the respiratory and olfactory epithelia observed in the low and high dose groups is likely a dose-response effect. The autolytic changes observed in group IV clearly suggested that immediate fixation is required to preserve the integrity of the cells of the nasal epithelium. Further investigation is required to

determine with precision the lower threshold concentration capable of inducing cell damage in nasal epithelium. The topographical distribution of the lesions clearly pointed out the predilection of the gas to selectively injure certain parts of the epithelium lining the nasal passages. The selectivity of gases for specific areas of the nasal epithelium has been described in animals exposed to other respiratory irritants (Buckley et al, 1984). The reason why the most rostral and caudal sectors, as well as the medial compared to the lateral aspects of the nasal cavity were less affected by hydrogen sulphide requires further study.

It was not possible to corroborate the polioencephalomalacia-like lesion described in monkeys exposed to a high dose (700 mg m^{-3}, 500 ppm) of hydrogen sulphide for only a 22 minute period (Lund & Wieland 1966).

In spite of the broad public and political concern for the potential health effects of hydrogen sulphide in the industrial, urban and natural ecosystems, few well controlled studies of the experimental and comparative pathology of hydrogen sulphide toxicity have been carried out. The dose-lesion relationship and sequence of events of the effect of hydrogen sulphide on the respiratory epithelium requires further work.

Emphasis should also be placed on the possible combined effect of inhaled hydrogen sulphide with naturally occurring diseases of the respiratory and cardiovascular systems, and on the effects of mixtures of airborne sulphur-containing compounds. The technical capability

for generating controlled and repeatable toxic atmospheres needs to be further refined, particularly for long term exposure of experimental animals at low or very low concentrations. Future work should not only investigate the short term effect of high doses, but also explore the more subtle chronic effects of the low concentrations often found in the industrial and natural environments.

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6.0 APPENDICES

6.1 APPENDIX A

ACCESSION NUMBER IDENTIFICATION

Animal Number	Accession Number	Animal Number	Accession Number
101	0001-85	301	0025-85
102	0002-85	302	0026-85
103	0003-85	303	0027-85
104	0004-85	304	0028-85
105	0005-85	305	0029-85
106	0006-85	306	0030-85
107	0007-85	307	0031-85
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206	0018-85	406	0042-85
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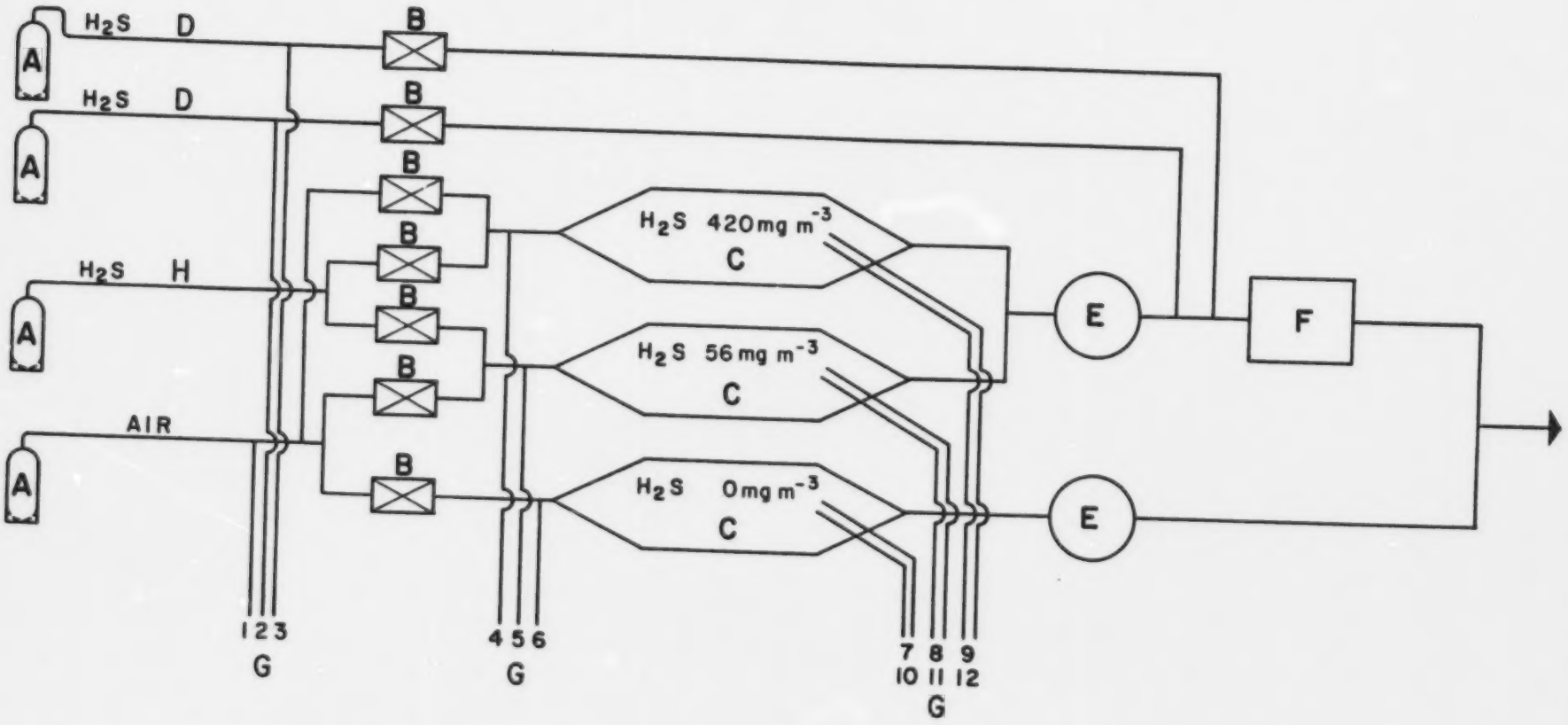
212	0024-85	412	0048-85



LEGEND

A	GAS CYLINDER	E	VACUUM PUMP
B	FLOW CONTROLLER	F	SCRUBBER
C	EXPOSURE CHAMBER	G	SAMPLE LINE
D	CALIBRATION STANDARD, HYDROGEN SULPHIDE	H	HYDROGEN SULPHIDE, 99.5 % C.P.

GENERAL SCHEMATIC OF INHALATION EXPOSURE SYSTEM

TABLE 1. Experimental design for rats exposed for six hours to an atmosphere of 0, 56 or 420 mg m^{-3} hydrogen sulphide.

Group	Treatment	Number of rats/post-exposure time		
		0 h	18 h	42 h
I	Room Control	4	4	4
II	Chamber Control*	4	4	4
III	H_2S 56 mg m^{-3}	4	4	4
IV	H_2S 420 mg m^{-3}	4	4	4

* compressed air

TABLE 2. Effect of hydrogen sulphide on body weight of rats exposed to 0, 56 or 420 mg m^{-3} hydrogen sulphide.

	Body weight, gm (Mean ± SD)			
Time Period	Room control	Chamber Control	Hydrogen sulphide, 56 mg m^{-3}	Hydrogen sulphide, 420 mg m^{-3}
Acclimation 1st week	139.3± 7.0(12)	141.3± 6.5(12)	142.9± 9.4(12)	144.6± 7.6(12)
Acclimation 2nd week	185.0±14.3(12)	190.5±10.2(12)	195.8±13.1(12)	199.3± 9.8(12)
Weight gain/day	6.5± 1.7	6.9± 0.8	7.5± 0.7	7.5± 0.8
Exposure day	223.1±19.0(12)	237.1±13.0(12)	245.5±15.5(12)	246.0±11.5(12)
0 h after exposure	NA	228.0±12.2(12)	226.1±14.6(12)	228.1±11.6(12)
Weight gain in 6 h	NA	-9.0± 1.8	-19.3± 3.0	-17.9± 4.4
18 h after exposure	238.8±21.7(8)	244.2±10.0(8)	230.7±14.3(8)	NA
Weight gain in 18 h	8.0± 1.6	14.6± 2.9	4.5± 2.6	NA
42 h after exposure	242.0±11.2(4)	242.0±10.3(4)	239.5±16.4(4)	NA
Weight gain in 42 h	4.2± 5.7	7.0± 3.6	11.5± 3.1	NA
Total weight gain since exposure	NA	20.5± 4.3	16.7± 2.7	NA

NA = not available
Number in parentheses is number of animals weighed.

TABLE 3. Histopathological changes in the nasal epithelium of rats exposed to 0, 56 or 420 mg m^{-3} hydrogen sulphide.

Time	Room Control	Chamber Control	56 mg m^{-3}	420 mg m^{-3}
00 hours	(01) focal rhinitis [2]*	(15) NAF	(29) necrosis [2,3]	(37) necrosis [3,4]
00 hours	(02) NAF	(20) NAF	(31) necrosis [2,3]	(38) necrosis [3,4]
00 hours	(04) NAF	(23) NAF	(32) necrosis [3,4]	(39) necrosis [3,4]
00 hours	(05) NAF	(24) NAF	(34) necrosis [2,3]	(40) necrosis [3,4]
				(41) necrosis [3,4]
				(42) necrosis [3,4]
				(43) necrosis [3,4]
				(44) necrosis [3,4]
				(45) necrosis [3,4]
				(46) necrosis [3,4]
				(47) necrosis [3,4]
				(48) necrosis [3,4]
18 hours	(03) NAF	(14) focal rhinitis [1]*	(26) necrosis, neutrophils [2,3]	
18 hours	(08) NAF	(16) NAF	(28) necrosis, neutrophils [1,2]	
18 hours	(10) focal rhinitis [1]*	(18) NAF	(35) necrosis, neutrophils [2,3]	
18 hours	(11) NAF	(19) NAF	(36) necrosis, neutrophils [2,3]	
42 hours	(06) focal rhinitis [2]*	(13) NAF	(25) reparation	
42 hours	(07) NAF	(17) NAF	(27) reparation	
42 hours	(09) NAF	(21) NAF	(30) reparation	
42 hours	(12) NAF	(22) NAF	(33) reparation	

* Local erosive lesion distinct to the necrosis observed in rats exposed to hydrogen sulphide.
NAF = no abnormal finding
Numbers in parentheses are rat identification numbers.
All sections of the 420 mg m^{-3} group showed some degree of autolysis.

TABLE 4. Number of rats per group with pulmonary oedema* following exposure to 0, 56 or 420 mg m^{-3} hydrogen sulphide.

Time	Room Control	Chamber Control	H_2S 56 mg m^{-3}	H_2S 420 mg m^{-3}
0	0/4**	0/4	4/4	12/12
18	0/4	0/4	0/3***	NA
42	0/4	0/4	0/4	NA

* includes perivascular and/or alveolar oedema
** number of rats affected/number of rats examined
*** one set of lungs not available
NA = not available.

TABLE 5. Rats with lymphorrhexis in either lymph node or thymus* following exposure to 0, 56 or 420 mg m^{-3} hydrogen sulphide.

Time	Room Control	Chamber Control	H_2S 56 mg m^{-3}	H_2S 420 mg m^{-3}**
0 hours	(01) -	(15) ++	(29) +	(37) +
0 hours	(02) -	(20) -	(31) +	(38) +++
0 hours	(04) +	(23) -	(32) +++	(39) ++
0 hours	(05) -	(24) -	(34) +	(40) +
				(41) +
				(42) -
				(43) +
				(44) +
				(45) +
				(46) +
				(47) +
				(48) +
18 hours	(03) -	(14) -	(26) NA	
18 hours	(08) -	(16) -	(28) +	
18 hours	(10) +	(08) +++	(35) -	
18 hours	(11) ++	(19) ++	(36) ++	
42 hours	(06) ++	(13) -	(25) +	
42 hours	(07) -	(17) -	(27) -	
42 hours	(09) -	(21) -	(30) -	
42 hours	(12) -	(22) -	(33) -	

- negative + mild ++ moderate +++ severe
NA = tissues not available
*Since lymph nodes were not available in all rats, the presence or absence of lymphorrhexis was determined in some animals in thymus only.
**All rats in this group were dead at the end of hydrogen sulphide exposure.
Numbers in parenthesis are rat identification numbers

TABLE 6. Incidental findings in rats exposed to 0, 56 or 420 mg m^{-3} hydrogen sulphide.

Rat #	Group	Lesion
01	I (room control)	Focal pancreatic hyperplasia and fibrosis
01	I	Focal erosive rhinitis
10	I	Focal erosive rhinitis
14	II (chamber control)	Focal erosive rhinitis
14	II	Focal mineralization of Peyer's patches
18	II	Focal atelectasis
22	II	Prostatic hyperplasia
31	III (56 mg m^{-3} H_2S)	Focal hepatic necrosis
35	III	Focal hepatic necrosis; Focal myositis
39	IV (420 mg m^{-3} H_2S)	Prostatic hyperplasia
44	IV	Focal hepatic necrosis
46	IV	Prostatic hyperplasia